Product Information

Annexin V Conjugates, Azide-Free and Lyophilized

Catalog no.	Conjugate	Unit Size	Ex/Em (nm)
29012R-5ug	Annexin V, CF®350 Conjugate	5 ug	347/448
29009R-5ug	Annexin V, CF®405M Conjugate	5 ug	408/452
29083R-5ug	Annexin V, CF®450 Conjugate	5 ug	450/538
29005R-5ug	Annexin V, CF®488A Conjugate	5 ug	490/515
29004R-5ug	Annexin V, CF®555 Conjugate	5 ug	555/565
29010R-5ug	Annexin V, CF®568 Conjugate	5 ug	562/583
29085R-5ug	Annexin V, CF®583R Conjugate	5 ug	586/609
29011R-5ug	Annexin V, CF®594 Conjugate	5 ug	593/614
29008R-5ug	Annexin V, CF®633 Conjugate	5 ug	630/650
29014R-5ug	Annexin V, CF®640R Conjugate	5 ug	642/662
29003R-5ug	Annexin V, CF®647 Conjugate	5 ug	650/665
29069R-5ug	Annexin V, CF®660R Conjugate	5 ug	663/682
29007	Annexin V, CF®680 Conjugate	25 ug	681/698
29070	Annexin V, CF®680R Conjugate	25 ug	680/701
29082	Annexin V, CF®700 Conjugate	25 ug	699/737
29006	Annexin V, CF®750 Conjugate	25 ug	755/777
29046	Annexin V, CF®770 Conjugate	25 ug	770/797
29047	Annexin V, CF®790 Conjugate	25 ug	784/806
29078	Annexin V, CF®800 Conjugate	25 ug	797/816

Visit www.biotium.com to view full dye spectra.

Storage and Handling

Store at 4°C and protect from light. Lyophilized solids are stable for at least 6 months from date of receipt when stored as recommended.

Preparation and Storage of Stock Solutions

To prepare stock solutions, dissolve in PBS or other buffer at 50 ug/mL. For 5 ug size, add 100 uL buffer to the vial; for 25 ug size, add 0.5 mL buffer to the vial; mix gently to dissolve. Note: Due to the small amount in the vial, lyophilized conjugates may not be visible. Stock solutions may be very pale or colorless depending on the conjugate.

Stock solutions can be sterilized by 0.2 um filtration, we recommend using Mini Syringe Filters for filtration of small volumes (see related products). Solutions are stable for up to a week when stored at 4°C, protected from light. For longer term storage, single-use aliquots of the 50 ug/mL stock can be stored at -20°C, although freezing can result in lower fluorescence. BSA can be added at 1 mg/mL final concentration to stabilize stock solutions during freezing. Aliquots should not be refrozen after thawing. Do not store dilute solutions of Annexin V in PBS.

Product Description

Fluorescent conjugates of Annexin V can be used to label apoptotic cells. The human anticoagulant Annexin V is a 35-36 kDa, Ca^{2+}-dependent phospholipid-binding protein with high affinity for phosphatidylserine (PS). In viable cells, PS is located on the inner leaflet of the cytoplasmic membrane. In apoptotic cells, PS translocates from the inner to the outer leaflet of the plasma membrane, where it can bind to fluorescent Annexin V for detection by microscopy or flow cytometry. Annexin V conjugates commonly are provided in solution with azide as a preservative for end-point staining assays. While end-point staining in Annexin Binding Buffer gives optimal results, staining also can be performed in complete cell culture medium. Staining in culture medium can avoid cell loss due to washing and buffer changes, and also allows apoptosis to be monitored over time in culture.

Our azide-free CF® Dye Annexin V Conjugates are supplied as lyophilized solids with no azide or other preservatives that might be incompatible with live cell imaging or *in vivo* studies. After dissolving in buffer, the conjugates can be added to cell culture medium for live cell staining. Near-infrared CF® Dye Annexin V Conjugates (CF®680 through CF®800) are compatible with NIR small animal *in vivo* imaging.

CF® dyes are superior to Alexa Fluor® dyes and Cy® dyes for protein labeling by having combined advantages in brightness, photostability, specificity, and novel features ideal for *in vivo* imaging. We also offer Annexin V conjugated to CF® dyes, biotin, R-PE and other labels in solution with azide as a preservative for end-point staining assays. See Related Products or visit www.biotium.com for more information about our apoptosis probes, including NucView® Caspase-3 Substrates for real-time monitoring of caspase activity in intact cells in real time.

Staining Protocols

Considerations for Annexin V staining

We provide example protocols below for no-wash staining with Annexin V in cell culture medium. Annexin V binding is calcium dependent, and should be performed using buffer or medium containing calcium for all incubation and wash steps. For end-point assays, we recommend using 1X Annexin V Binding Buffer (see related products) or other serum-free buffer containing 2.5 mM calcium. Different culture media vary in calcium concentration, which may require optimization for Annexin V binding. Note that buffers with phosphate or serum-free media may be harmful to cells if calcium is added.

When possible, it is recommended to include a positive control with a treatment that is verified to induce apoptosis in your cell type. Staurosporine (see related products) can be used to induce apoptosis in many (but not all) cell types.

Staining cells for fluorescence microscopy by medium exchange

1. Induce apoptosis in cells by the desired method. Include a control sample of untreated cells.
2. Prepare Annexin V stock solution from lyophilized solid as described under Preparation and Storage of Stock Solutions.
3. Dilute azide-free Annexin V to a final concentration of 0.25 ug/mL in cell culture medium. For example, add 1 uL of 50 ug/mL Annexin V to 200 uL cell culture medium. Prepare enough staining solution to completely cover cells.

   Notes:
   1) The optimal staining concentration should be determined empirically.
   2) Other stains like our NucView® caspase substrates or dead cell nuclear stains can be included together with Annexin V.
4. Remove culture medium from cells and replace with medium containing Annexin V from step 3.

   Note: Floating dead cells in adherent cultures may be lost upon removal of medium. These cells may need to be collected and stained separately to accurately detect the number of apoptotic cells in the sample.

   Cells in suspension culture need to be pelleted by centrifugation (usually at 350 x g for 5 minutes), before exchanging medium and resuspending cells.
5. Incubate cells in the dark at 37°C for at least 15 minutes before imaging. Cells can be continuously incubated with Annexin V for at least 48 hours.

Staining cells for fluorescence microscopy by direct addition of 10X probe

This method is convenient because it does not require removal of cell culture medium, which can result in loss of detached apoptotic cells (see Figure 1). It also eliminates the need to centrifuge suspension cells before staining. Take care to gently mix the medium immediately after adding Annexin V solution to each sample to avoid uneven staining from transient high local probe concentration, or cell disruption by overly vigorous pipetting. Adding undiluted (50 ug/mL) Annexin V stock solution directly to cells is not recommended.

1. Induce apoptosis in cells by the desired method. Include a control sample of untreated cells.
2. Prepare Annexin V stock solution from lyophilized solid as described under Preparation and Storage of Stock Solutions.
3. Prepare a 10X Annexin V solution in culture medium. For example, to stain cells with a final concentration of 0.25 ug/mL Annexin V, prepare 10X Annexin V (2.5 ug/mL) by adding 1 uL of 50 ug/mL Annexin V to 20 uL medium.

   Notes:
   1) The optimal staining concentration should be determined empirically.
   2) Other stains like our NucView® caspase substrates or dead cell nuclear stains can be included together with Annexin V.
4. Without removing the culture medium from the cells, add 1/10 volume of 10X Annexin V solution to the medium on the cells. For example, for cells seeded in a 96-well plate in 100 uL per well, add 10 uL of 10X Annexin V to the medium in the well. Immediately mix by gently pipetting half the volume of the well up and down several times, taking care not to introduce bubbles. Scale all volumes proportionally for larger culture vessels.
5. Incubate cells in the dark at 37°C for at least 15 minutes before imaging. Cells can be continuously incubated with Annexin V for at least 48 hours.

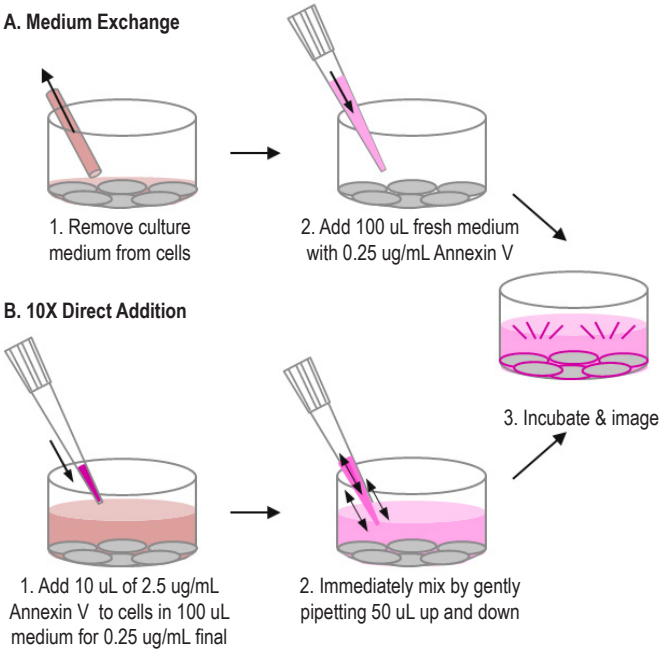

Figure 1. Example of medium exchange (A) vs. direct addition of 10X probe (B) for live cell imaging. Medium exchange results in uniform exposure of cells to probe, but floating dead cells may be lost during medium removal, and suspension cells must be pelleted by centrifugation. Direct addition of 10X probe avoids loss of cells, but care must be taken to mix immediately yet gently to avoid high transient probe concentration or disruption of cells by pipetting. Volumes shown are for staining cells in a 96-well plate with 100 uL medium per well; volumes should be scaled proportionally for different well sizes. The final concentration of Annexin V may require optimization for different detection systems.

Staining cells for flow cytometry

Note: for flow cytometry we recommend performing end-point staining in 1X Annexin Binding Buffer (see Related Products) or other serum-free buffer (e.g., HBSS buffer) containing 2.5 mM $CaCl_2$.

1. Induce apoptosis in cells by desired method. Include the following negative control samples:
   a. Untreated cells, unstained
   b. Apoptosis-induced cells, unstained
   c. Untreated cells, Annexin V stained
2. Adherent cells should be detached and placed in suspension before staining. Be sure to collect any detached apoptotic cells from the culture and include them in the sample.
3. Pellet cells by centrifugation (usually at 350 xg).
4. Remove supernatant and wash once by resuspending cells in PBS or binding buffer.
5. Pellet cells by centrifugation.
6. Remove all traces of supernatant and resuspend cells in 1X Annexin Binding Buffer at 2-3x10^6 cells/mL.
7. Aliquot 100 uL cells per flow cytometry tube.
8. Prepare Annexin V stock solution from lyophilized solid as described under Preparation and Storage of Stock Solutions.
9. Prepare a 10X Annexin V solution in buffer. For example, to stain cells with a final concentration of 0.5 ug/mL Annexin V, prepare 10X Annexin V (5 ug/mL) by adding 1 uL of 50 ug/mL Annexin V to 9 uL buffer.

   Note: 0.5 ug/mL is recommended as a starting point for initial testing. The optimal staining concentration should be determined empirically.
10. Add 10 uL of 10X Annexin V solution per tube and immediately mix by gentle shaking or low-speed vortexing.

    Note: Other stains like our NucView® caspase substrates or dead cell nuclear stains can be included together with Annexin V.
11. Incubate at room temperature for at least 15 minutes, protected from light.
12. Add 400 uL of binding buffer to each tube and analyze the cells by flow cytometry within 1 hour of staining.

Fixation after staining

Annexin V cannot be used to stain fixed cells or tissues. After staining with Annexin V and washing, cells can be fixed with 2% formaldehyde. Annexin V staining is calcium dependent, we recommend including 2.5 mM calcium in the buffer and formaldehyde solution used for washing and fixation. Annexin V binds to a phospholipid in the plasma membrane, therefore staining is not compatible with alcohol fixation or detergent permeabilization.

Related Products

Catalog Number	Product
99902	5X Annexin Binding Buffer (15 mL)
22025	Mini Syringe Filters (Set of 5)
22026	Mini Syringe Filtration Kit (for 5 Samples)
10405	NucView® 405 Caspase-3 Substrate, 1 mM in DMSO
10402	NucView® 488 Caspase-3 Substrate, 1 mM in DMSO
10403	NucView® 488 Caspase-3 Substrate, 1 mM in PBS
10406	NucView® 530 Caspase-3 Substrate, 1 mM in DMSO
30067	Dual Apoptosis Assay Kit with NucView® 488 Caspase-3 Substrate & CF®594 Annexin V
30076	Dual Apoptosis Assay Kit with NucView® 488 Caspase-3 Substrate & CF®640R Annexin V
30062	NucView® 488 and MitoView™ 633 Apoptosis Kit
30072	NucView® 488 and RedDot™2 Apoptosis and Necrosis Kit
30065	Apoptosis & Necrosis Quantitation Kit Plus
30066	Apoptotic, Necrotic & Healthy Cells Quantitation Kit Plus
30060	CF®488A Annexin V and 7-AAD Apoptosis Kit
30061	CF®488A Annexin V and PI Apoptosis Kit
40061	RedDot™2 Far-Red Nuclear Stain (for dead or fixed cells)
40083	NucSpot® 470 Green Nuclear Stain (for dead or fixed cells)
40017	Propidium Iodide, 1 mg/mL in water
40084	7-AAD Solution, 1 mg/mL
30001	JC-1 Mitochondrial Membrane Detection Kit
70005	TMRE 2 mM in DMSO
30063	CF®488A TUNEL Assay Apoptosis Detection Kit
30064	CF®594 TUNEL Assay Apoptosis Detection Kit
30074	CF®640R TUNEL Assay Apoptosis Detection Kit
80027	PathoGreen™ Histofluorescent Stain
32010	Live-or-Dye NucFix™ Red Staining Kit
32002-32009	Live-or-Dye™ Fixable Viability Staining Kits
00025	Staurosporine
22020	10X Phosphate-Buffered Saline (PBS)
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative

A full selection of CF® dye labeled products including secondary antibodies, streptavidin and anti-biotin antibodies, antibody labeling kits, and other bioconjugates such as phalloidins, lectins, and α-bungarotoxins are also available. Please visit the Biotium website at www.biotium.com for details.